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X-RAY CRYSTAL STRUCTURE AND CONFORMATIONAL ANALYSIS OF N-(3-DIMETHYLAMINOPROPYL)-N-(ETHYLAMINOCARBONYL)-6-(2-PROPENYL)ERGOLINE-8β-CARBOXAMIDE (CABERGOLINE): COMPARISON WITH BROMOCRIPTINE AND LISURIDE AND A HYPOTHESIS FOR ITS HIGH DOPAMINERGIC ACTIVITY

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Summary — The crystal structure of Cabergoline, a potent and long lasting prolactin lowering agent interacting with the D2 dopamine receptors, has been determined by X-ray diffraction data. The structural data represent the starting point for a computational study, where the molecular mechanics approach was used to explore the motion of all unconstrained torsion angles. Two different conformations related to energetic minima have been found, one of them in agreement with the experimental crystal structure. Both conformations are shape compared with Bromocriptine and Lisuride, two dopaminergic ergoline derivatives with C-8β and C-8α substituents of the ergoline ring, respectively . We observe that the C-8β Cabergoline assumes the overall three-dimensional features of C-8-α-ergolines in one of its low-energy conformations.

INTRODUCTION

The conformational analysis and the structure determination of pharmacologically active compounds are critical for a rational design of new drugs and for the understanding of appropriate drug-receptor interaction at the molecular level[1] in the quest for improved therapeutic profile.

The natural ergot alkaloid and semisynthetic ergoline derivatives are a class of compounds which show broad pharmacological activity[2] in central nervous system on dopamine, adrenaline and serotonine receptors[3] and in peripheral sites as the autonomic nerve endings in the cardiovascular system[4]. Both potency and selectivity of these compounds, where the ergoline skeleton represents the structural invariant moiety, strongly depend on the different substituents in position C8.

The N-(3-dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-propenyl) ergoline-8β-carboxamide, namely Cabergoline[5], was synthesized at Pharmacia Laboratory during a project aimed at identifying therapeutically active ergoline derivatives. The compound shows very interesting pharmacological agonistic activity on the D2 dopamine receptor, possessing improved therapeutic characteristics with respect to Bromocriptine and Lisuride[6,7]. These latter bear, respectively, C-8β and C-8α substituents and are employed against Parkinson disease and hyperprolactinemia. Cabergoline was demonstrated to have, besides a potent dopaminergic activity, long lasting properties when compared to Bromocriptine and Lisuride[8].

We have previously reported the crystal structures as well as some theoretical studies on various ergolines[9,10]; here we present the solid-state structure of Cabergoline as determined by X-ray diffraction methods, together with its conformational analysis.

EXPERIMENTAL SECTION

The crystals of Cabergoline were grown as transparent plates from ethylic ether. The crystallographic data are $C_{26}H_{37}N_5O_2$, Mr = 451.5 monoclinic, $P2_1$, a = 9.046(4), b = 12.250(5), c = 11.781(8) Å, β = 93.91(5), V = 1302.4 (Å)^3, Z = 2, Dx = 1.15 Mg m^{-3}. Cell dimensions were obtained from 25 reflections ($7^\circ \leq \theta \leq 11^\circ$); data collection was performed on an Enraf-Nonius CAD4-diffractometer, graphite monocromatized MoKα radiation (λ = 0.71069 Å), μ = 0.07 mm^{-1}, F(000) = 488, T = 293 K, $\omega/2\theta$ scan, 2θ max 50°, scan speed 5°sec^{-1}, scan width 0.8° + 0.35 tg θ, background measured in stationary mode, 0.5 times the peak scan time, 2517 collected reflections ($h = -8 \div 8$; $k = 0 \div 11$; $l = 0 \div 11$), 1858 unique reflections (R_{int} = 0.02) 780 of which with $I > 2.5\ \sigma(I)$, three standard reflections (231, 131, 210), no significant intensity variation; no absorption or extinction correction applied. The structure of the compound was solved by program MITHRIL[11], which allowed the location of all the heavy atoms. Full refinement based on F by least squares methods was performed using SHELXL93[12]. Number of parameters refined 143. Values of R = 0.062 and

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wR = 0.065. Atomic scattering factors taken from SHELXL3. Max $\Delta/\sigma = 0.1$ referring to thermal parameters. Maximum positive and negative electron density in final difference Fourier maps 0.23 to -0.22 e $Å^{-3}$. Programs PLATON[13] and SCHAKAL[14] were used for geometrical calculations and graphics.

The complete conformational analysis of Cabergoline by the software SYBYL[15] has adopted the TRIPOS force field for computing the conformational energies. Hydrogen atoms at standard bond lengths and angles have been added to the heavy atoms parameters; atomic charges were determined with the semiempiric molecular orbital software MOPAC[16]. After an energy minimisation of the starting molecule the torsional angles, excluding the $-CH_3$ and $-N(CH_3)_2$ terminal groups, were rotated of 15° stepwise. The two possible minima thus found were superimposed on the ergoline ring of the structures of Bromocriptine and Lisuride, the former extracted from the Cambridge Crystallographic Data Base[17] and the latter built at the computer by molecular modelling.

Complete X-ray crystallographic data have been deposited at the Cambridge Crystallographic Data Centre.

RESULTS AND DISCUSSION

The X-ray atomic coordinates are reported in Table I. Bond distances and angles, listed in Table II, are comparable and similar, for the ergoline moiety, to the values found in analogues[9,10].

Fig.1 shows a plot of Cabergoline together with the atomic labelling. In addition to the van der Waals interaction, the crystal packing is engineered by two H-bonds: an intramolecular N(25)-H(25)...N31 2.870 (Å) and an intermolecular one N(1)-H(1)....N6(i) 2.946 (Å), where (i) is the translation -x, 0.5+y, 2-z; the

TABLE I - Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for Cabergoline...

	x	y	z	U(eq)
N(1)	−1324(7)	6269(6)	10593(5)	53(2)
C(2)	−30(8)	5720(7)	10855(6)	48(2)
C(3)	276(8)	5044(6)	10001(6)	41(2)
C(4)	1506(9)	4255(7)	9797(7)	53(2)
C(5)	1602(8)	4049(7)	8503(6)	42(2)
N(6)	2630(6)	3155(5)	8271(5)	41(2)
C(7)	2777(9)	3053(7)	7033(7)	55(2)
C(8)	1375(9)	2699(7)	6435(7)	54(2)
C(9)	162(9)	3555(7)	6646(7)	50(2)
C(10)	67(8)	3757(7)	7906(6)	46(2)
C(11)	−1066(8)	4633(6)	8115(6)	39(2)
C(12)	−2310(9)	4890(8)	7412(7)	58(2)
C(13)	−3319(10)	5701(8)	7786(7)	68(3)
C(14)	−3103(9)	6202(7)	8859(7)	53(2)
C(15)	−1882(8)	5924(6)	9538(6)	41(2)
C(16)	−878(8)	5150(6)	9138(6)	41(2)
C(17)	4081(9)	3294(7)	8865(7)	58(2)
C(18)	5000(12)	2246(9)	8746(8)	88(3)
C(19)	6377(14)	2249(13)	8608(10)	130(5)
C(20)	1482(10)	2557(8)	5156(8)	62(3)
O(21)	2552(7)	2913(6)	4733(5)	78(2)
N(22)	301(8)	2136(6)	4540(6)	58(2)
C(23)	−804(10)	1494(8)	4935(8)	67(3)
O(24)	−550(8)	971(6)	5829(6)	92(2)
N(25)	−2085(8)	1477(6)	4363(6)	70(2)
C(26)	−3255(10)	799(9)	4720(8)	78(3)
C(27)	−4214(11)	1397(12)	5453(11)	145(5)
C(28)	353(9)	2312(8)	3285(7)	73(3)
C(29)	10(11)	3440(8)	2901(8)	75(3)
C(30)	−1468(10)	3867(9)	3129(9)	84(3)
N(31)	−2745(10)	3195(8)	2729(8)	92(3)
C(32)	−2802(18)	3124(14)	1525(13)	159(6)
C(33)	−4108(16)	3616(14)	3011(12)	150(6)

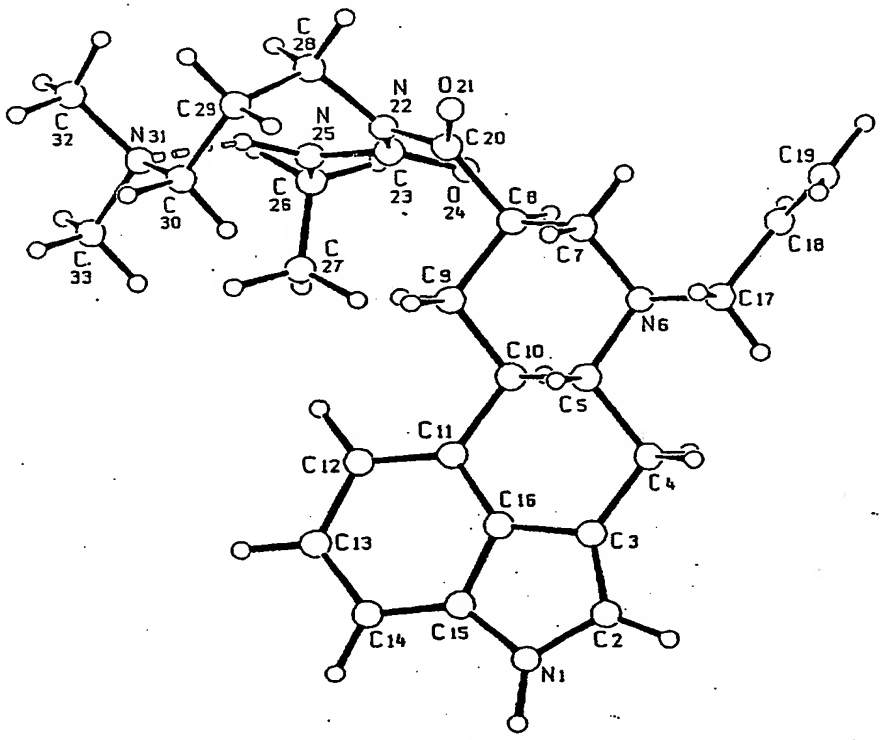

Fig. 1: X-ray molecular conformation of Cabergoline showing the crystallographic labelling. The dashed line depicts the hydrogen bond between N(25)-H(25) and N(31).

TABLE II - Bond lengths [Å] and angles [°] for Cabergoline.

N(1)-C(2)	1.37(1)	C(13)-C(14)	1.41(1)
N(1)-C(15)	1.38(1)	C(14)-C(15)	1.36(1)
C(2)-C(3)	1.35(1)	C(15)-C(16)	1.41(1)
C(3)-C(16)	1.41(1)	C(17)-C(18)	1.54(1)
C(3)-C(4)	1.50(1)	C(18)-C(19)	1.27(1)
C(4)-C(5)	1.55(1)	C(20)-O(21)	1.20(1)
C(5)-N(6)	1.47(1)	C(20)-N(22)	1.35(1)
C(5)-C(10)	1.55(1)	N(22)-C(23)	1.38(1)
N(6)-C(17)	1.45(1)	N(22)-C(28)	1.50(1)
N(6)-C(7)	1.48(1)	C(23)-O(24)	1.24(1)
C(7)-C(8)	1.47(1)	C(23)-N(25)	1.30(1)
C(8)-C(20)	1.53(1)	N(25)-C(26)	1.43(1)
C(8)-C(9)	1.55(1)	C(26)-C(27)	1.46(1)
C(9)-C(10)	1.51(1)	C(28)-C(29)	1.48(1)
C(10)-C(11)	1.51(1)	C(29)-C(30)	1.48(1)
C(11)-C(16)	1.36(1)	C(30)-N(31)	1.47(1)
C(11)-C(12)	1.39(1)	N(31)-C(33)	1.40(2)
C(12)-C(13)	1.44(1)	N(31)-C(32)	1.42(2)
C(2)-N(1)-C(15)	108.0(6)	C(14)-C(15)-N(1)	133.4(7)
C(3)-C(2)-N(1)	110.5(7)	C(14)-C(15)-C(16)	119.0(7)
C(2)-C(3)-C(16)	107.5(6)	N(1)-C(15)-C(16)	107.6(6)
C(2)-C(3)-C(4)	134.9(7)	C(11)-C(16)-C(3)	129.5(7)
C(16)-C(3)-C(4)	117.5(7)	C(11)-C(16)-C(15)	124.2(7)
C(3)-C(4)-C(5)	110.7(6)	C(3)-C(16)-C(15)	106.3(6)
N(6)-C(5)-C(10)	107.5(6)	N(6)-C(17)-C(18)	109.5(7)
N(6)-C(5)-C(4)	112.4(6)	C(19)-C(18)-C(17)	123.4(12)
C(10)-C(5)-C(4)	111.9(6)	O(21)-C(20)-N(22)	123.0(9)
C(17)-N(6)-C(5)	112.5(6)	O(21)-C(20)-C(8)	118.4(8)
C(17)-N(6)-C(7)	110.2(6)	N(22)-C(20)-C(8)	118.2(8)
C(5)-N(6)-C(7)	110.2(6)	C(20)-N(22)-C(23)	126.9(8)
N(6)-C(7)-C(8)	111.4(6)	C(20)-N(22)-C(28)	113.4(7)
C(7)-C(8)-C(20)	113.3(7)	C(23)-N(22)-C(28)	119.3(7)
C(7)-C(8)-C(9)	108.6(7)	O(24)-C(23)-N(25)	122.6(9)
C(20)-C(8)-C(9)	109.2(7)	O(24)-C(23)-N(22)	119.0(9)
C(10)-C(9)-C(8)	110.9(7)	N(25)-C(23)-N(22)	118.4(8)
C(9)-C(10)-C(11)	111.0(6)	C(23)-N(25)-C(26)	120.5(8)
C(9)-C(10)-C(5)	111.8(6)	C(27)-C(26)-N(25)	111.4(9)
C(11)-C(10)-C(5)	110.9(6)	C(29)-C(28)-N(22)	114.6(8)
C(16)-C(11)-C(12)	118.1(7)	C(28)-C(29)-C(30)	116.7(9)
C(16)-C(11)-C(10)	115.3(7)	N(31)-C(30)-C(29)	116.4(9)
C(12)-C(11)-C(10)	126.4(7)	C(33)-N(31)-C(32)	106.8(11)
C(11)-C(12)-C(13)	118.6(8)	C(33)-N(31)-C(30)	113.8(10)
C(14)-C(13)-C(12)	121.6(8)	C(32)-N(31)-C(30)	109.2(10)
C(15)-C(14)-C(13)	118.4(8)		

Table III - Relevant torsional angles (°), energies (E) and root mean square deviations (RMS) of the heavy atoms of conformers Ia and Ib of Cabergoline versus the crystallografic data.

	X-ray	Ia	Ib
C(7) - C(8) - C(20) - N(22)	173.4	172.5	150.1
C(8) - C(20) - N(22) - C(23)	−21.2	−9.1	179.0
C(20) - N(22) - C(23) - N(25)	155.2	162.9	−0.4
C(20) - N(22) - C(28) - C(29)	−73.0	−75.1	−86.8
N(22) - C(28) - C(29) - C(30)	−60.1	−54.6	−62.8
C(28) - C(29) - C(30) - N(31)	−51.3	−56.1	−173.0
C(23) - N(25) - C(26) - C(27)	91.2	77.5	78.4
E (Kcal/mole)		−3.23	−3.78
RMS (Å)		0.200	3.117

respective H-bond angles are 157.4° and 165.9°. The presence of the intermolecular hydrogen bond N(1)-H(1)...N(6) throws further light on the role of these two sites in the molecular recognition of this compound. In fact, the basic pharmacophore of the ergoline ring in order to attain dopaminergic activity was inferred to include both N(1)H and N(6)[18], together with the phenyl group of the indole, where N(1)H forms a hydrogen bond as a donor with the receptor active site and N(6) plays its role as an acceptor or forms a ionic bond, when protonated.

However, the resulting three dimensional structure represents only one of the possible conformations: nothing is known about the bioactive form that allows the receptor, as in the case of D2 receptor, to be coupled with G proteins[19] and consequently to elicit a biological response. For this reason we have decided to sample every possible theoretical conformation starting from the crystallographic structure for comparison with other dopaminergic compounds of the ergoline class, such as Bromocriptine and Lisuride. The conformational analysis based on torsional angles gives two different minima : torsional angles, energies and RMS (root mean square) deviations of the heavy atoms with respect to the X-ray data are reported in Tab.III. The two conformations (Ia and Ib) are sketched in Fig. 2. The first one, Ia, is pratically superimposable to the crystal structure with RMS deviation of 0.2 Å, while Ib is different and it is characterized by a strong intramolecular hydrogen bond between N(25)-H(25) and O(21), giving rise to a six-membered ring, which explains the slightly lower conformational energy of Ib over Ia. Moreover, in Ib the $-(CH_2)_3N(CH_3)_2$ moiety, not being involved in the hydrogen bond observed in the crystal structure, is considerably free of moving with a favourable entropic contribution.

These considerations are obviously valid for the shape of Cabergoline in the crystal packing environment and in vacuum, while nothing is certain about the preferred conformations in solution or at the receptor site. In solution Cabergoline might exist as a mixture of the two forms Ia and Ib with a yet unknown ratio and the bioactive conformation is close either to Ia or Ib. Actually, it was recently suggested[20] that the powerful dopaminergic properties of Cabergoline could be explained by one bioactive structure possessing a combination of C-8β (equatorial) substitution and C-8α (axial) shape, respectively present in the aforementioned compounds Bromocriptine and Lisuride. We have superimposed the ergoline moieties containing the dopaminergic pharmacophore, i.e. N(1H), N(6) and phenyl ring of all the structures, on Bromocriptine and observed the topology and steric encumbrance of the C8 substituents in the compounds under study.

The shape of conformer Ia, very close to the crystal structure, shows (fig. 2) the C8 substituent as β type, like in Bromocriptine, while in the case of conformer Ib it is evident that the $(CH_2)_3N(CH_3)_2$ moiety

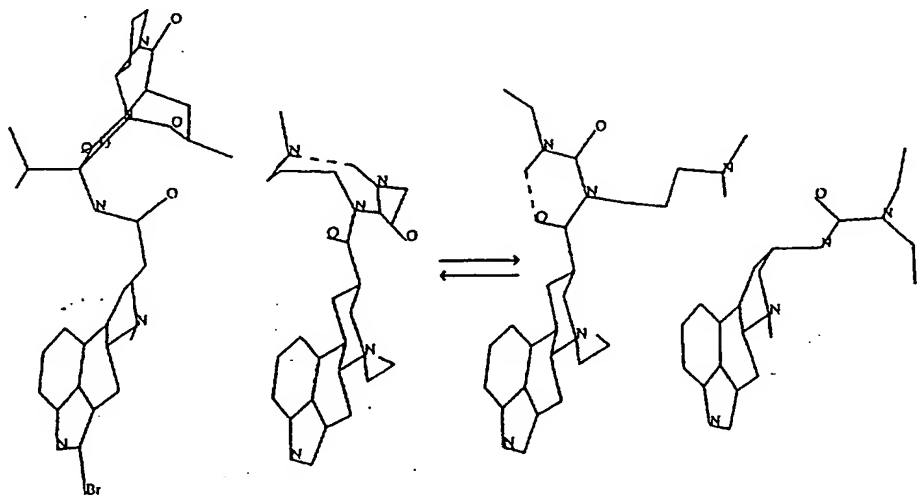

Fig. 2: Bromocriptine and Lisuride together with the two stable conformations, Ia and Ib, of Cabergoline. The dashed lines in Ia and Ib represent the hydrogen bonds N(25)-H(25)...N(31) and N(25)-H(25)...O(21), respectively.

protrudes in the same space region of the C-8α substituent of Lisuride. Therefore we cannot establish unequivocally what the active form of Cabergoline could be. However, we suggest that Cabergoline is present at the receptor with two different, active conformations in equilibrium. One of them is interacting at the binding sites of C-8β ergolines and the other at those of C-8α of the D2 receptor. The hypothesis of two active conformations, which has never been put out before, may be the explanation for the peculiar pharmacological features of Cabergoline.

CONCLUSION

The explanation for the remarkable potency of Cabergoline with respect to other dopaminergic ergoline derivatives of similar pharmacological profile may be possibly linked to an equilibrium of two conformations, both active upon the D2 dopamine receptor. These structures interact with the active site by means of the pharmacophore N(1)H, N(6) and phenyl ring with the right spatial stereochemistry; at the same time the C8 substituent, forming important secondary binding sites, may recognize the receptor pocket of C-8β or C-8α ergolines.

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